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Temporal change of the Baltic Sea–North Sea blue mussel hybrid zone over two decades

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Abstract

In a temporal comparison over 18 years, we documented changes in the position and structure of the North European blue mussel hybrid zone in the Öresund strait, between *Mytilus edulis* of the marine Kattegat and *Mytilus trossulus* of the brackish Baltic Sea. In 1987 the midpoint of the 140-km wide multilocus allozyme cline in shallow-water populations was estimated to be located half-way along the strait. In 2005, it was shifted 25 km towards the Baltic end of the Öresund, and was located near the fixed link (bridge) that was built across the strait meanwhile in the 1990s. The cline also appeared to have become narrower and the extent of hybridity among individuals decreased. Factors that theoretically can control the position and shape of a clinal hybrid zone involve environmental gradients between habitats that differentially favor the two hybridizing taxa, or barriers to geographical dispersal of the organism. We consider two alternative hypotheses to explain the movement of the mussel hybrid zone. (1) Environmental change related to climate warming: the more stenothermal *M. trossulus* was pushed out from the Öresund towards the cool Baltic by elevated temperatures. (2) Change of dispersal dynamics: the construction of the fixed link locally affected mussel dispersion which attracted the zone. We raise the question whether similar changes have taken place also in the other euryhaline taxa where genetic clines between Baltic vs. Kattegat populations occur.

Introduction

The Danish Straits in Northern Europe, comprising the Belts and the Öresund, make a transitory area that connects the relatively marine Kattegat (extension of the North Sea) and the pronouncedly brackish and cool inner Baltic Sea basin through narrow passageways (HELCOM 1996). The environmental transition over this area is known to be generally important for isolation and connectivity between populations of the Baltic vs. the Kattegat and the North Sea. Genetic clines coincident with the strong environmental gradients in the Straits have been documented in many euryhaline marine organisms (Johannesson and André 2006). These clines have mostly been interpreted as hybrid zones, either primary or secondary (e.g., Väinölä and Varvio 1989; Väinölä and Hvilsom 1991; Nielsen et al. 2004; Johannesson and André 2006).

Hybrid zones are areas of overlap and hybridization between closely related taxa. From the point of view of genetic characters, a typical hybrid zone is a multilocus S-shaped cline with a deficit of recombinant genotypes in the center. As a rule, such zones are associated with environmental gradients separating parental taxa and (or) with areas of low population density or other barriers to dispersal (Barton and Hewitt 1985). Under different evolutionary scenarios hybrid zones may be seen as transitional stages of processes leading to complete reproductive isolation between the hybridizing taxa (speciation), to break-up of partial reproductive barriers and fusion of taxa (speciation reversal), or to the formation of reproductive barriers between hybrid and parental

populations (hybrid speciation) (Arnold 1997; Abbott et al. 2013). In practice, however, no such changes in the dynamics of hybridization can be seen in hybrid zones over the periods they have been observed, and they are therefore referred to as stable hybrid zones (Barton and Hewitt 1985). Stable hybrid zones do not need to be spatially static; however, the geographical location and shape of a zone can change in response to changes in position of an underlying ecotone, or with change in the patterns of population density and dispersal dynamics of the species in the area (Barton and Hewitt 1985). Global climate change and more local anthropogenic disturbances can be important factors of hybrid zone dynamics (Buggs 2007). The question about the temporal stability and dynamics of hybrid zones at the entrance to the Baltic Sea has so far rarely been addressed [study of Simonsen and Strand (2010) is a rare exception].

The blue mussels *Mytilus* spp. are epibenthic broadcast spawning bivalves with a prolonged pelagic larval stage in their life cycles. They dominate in the shallow-water communities of many northern seas, including the Baltic (Seed and Suchanek 1992; Kautsky and Kautsky 1995). In the conventional view, the main *Mytilus* species in the boreal North Atlantic is *M. edulis* Linnaeus, 1758, whereas the originally Pacific *M. trossulus* Gould is also present in several regions, including north-eastern North America, and the Baltic Sea and several localities in Scotland, Norway and Northern Russia in Europe (McDonald et al. 1991; Beaumont et al. 2008; Väinölä and Strelkov 2011; Wenne et al. 2016). In spite of the old divergence of the species lineages (ca. 3.5 Ma, Vermeij 1991; Rawson and Hilbish 1998), hybridization between them occurs to varying degrees wherever their distributions overlap (Koehn 1991; Beaumont et al. 2008; Väinölä and Strelkov 2011; Wenne et al. 2016 and references therein). The Danish Straits are an area of secondary intergradation between *M. edulis* of the Kattegat and *M. trossulus* of the inner Baltic waters. Particularly, in the Öresund strait, a hybrid zone reflected in relatively steep parallel clines at a number of allozyme loci has been described. This zone has been suggested to be maintained by genetic selection against hybrids and by physiological selection against larvae entering a salinity regime different from their native habitat in the transition zone, which restricts the effective dispersal (Väinölä and Hvilsom 1991). The distinction of the Baltic and North Sea populations has also been documented in a large number of other, DNA-based genetic markers (e.g., Zbawicka et al. 2014; Fraïsse et al. 2016).

Although the origin and history of *M. trossulus* in the Baltic Sea are not known for certain, a credible hypothesis holds that it is an early post-glacial invader, representing a relict that persisted in the semi-isolated basin for millennia, in parapatric contact with the Kattegat *M. edulis*, and meanwhile evolved unique phenotypic and genetic traits, but also adopted some features by introgression (Väinölä and Hvilsom 1991; Väinölä and Strelkov 2011). The hybrid zone at the entrance of the Baltic would then be a rather old and presumably stable formation. It seems highly unlikely that such a long standing zone could experience any evolutionary dynamics (i.e., dynamics related to the process of reinforcement or erosion of reproductive isolation barriers) in the short term. This hypothesis could be addressed by direct observations in temporal comparisons over the zone.

The blue mussel intergradation in the Öresund was one of the first hybrid zones that was described in the marine realm. Considerable changes have occurred in the Öresund environment since the zone was discovered in the 1970s–1980s (Theisen 1978; Bulnheim and Gosling 1988; Väinölä and Hvilsom 1991). The Baltic Sea has been reported as one of the fastest-warming marine ecosystems in the context of global climate change (Belkin 2009; Lima and Wethey 2012). The monumental Öresund Bridge, or the Öresund Fixed Link was constructed in the late 1990s just across the zone. It has generally been thought that the link only had inessential impact on the marine environment (Stigebrandt 1992; Gray 2006). However, at the genetic level, the impacts of the link have not been addressed. There are either no studies on the impacts of the global climate change on the location of genetic clines at the entrance to the Baltic Sea. Our study addresses the stability and dynamics of

the hybrid zone between *M. edulis* and *M. trossulus* in the Öresund in a temporal comparison over a two-decade interval.

Materials and methods

Study area

The Öresund between the Swedish mainland and the Sjaelland (Zealand) island of Denmark, also known as the Sound, is an 80 km long and 4–40 km wide strait that connects the Kattegat in the north to the Baltic Sea in the south (Fig. 1a). The mean surface salinity is 10 PSU on the Baltic side and 17 PSU on the Kattegat side of the Öresund. Hydrodynamics is intense and local salinities vary temporally, but the main oceanographical break is at the Drogden Sill, which is the shallowest part of the strait (7 m deep), located in the southern part of the Öresund, towards the Baltic entrance (Nordenberg 1980; Fischer and Matthäus 1996). The Öresund Fixed Link is a carriageway across the Öresund, built in 1995–1999 at the Sill locality; it consists of an 8-km-long bridge between the Swedish coast and an artificial island, the 4-km-long island, and a 4-km tunnel to the Danish coast.

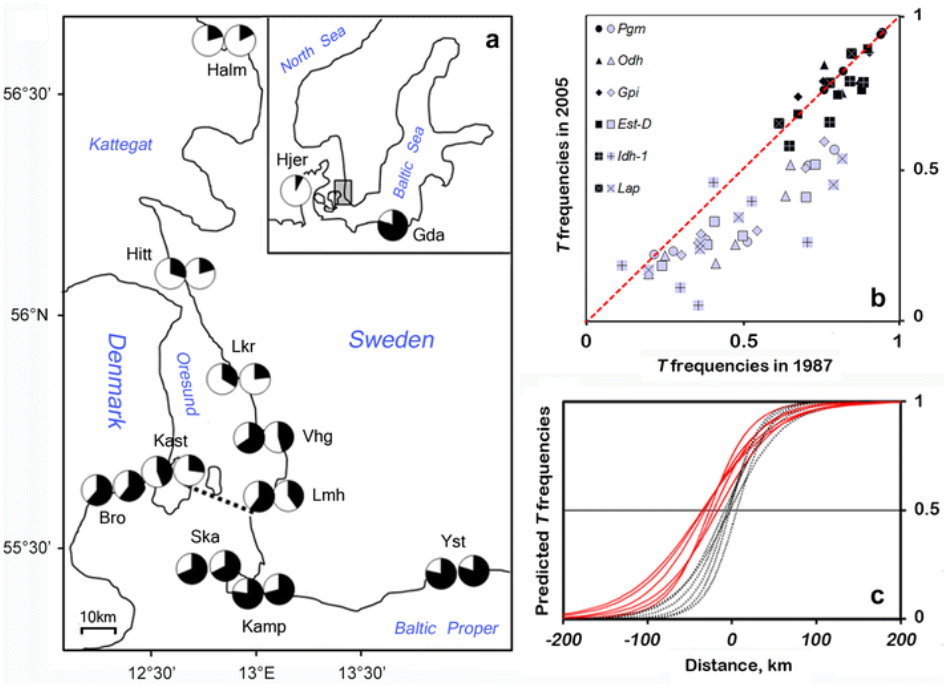

Fig. 1 Temporal changes in the genetic composition of *Mytilus* populations from the Öresund. (a) Map of the study area, the Öresund and surroundings. Sampling locality codes as in Table 1. Pie diagrams illustrate the (non-scaled) average allele frequencies from all six loci in temporal samples from 1987 (left) and 2005 (right). Black sector—*T* alleles, white sector—*E* alleles. (b) Scaled frequencies of *T* alleles at individual loci in samples from 2005 vs. 1987. For samples with no temporal change, points would fall on the diagonal. Points above the diagonal indicate an increase of *T* frequencies from 1987 to 2005, and vice versa. Samples north (to the Kattegat side) of the Bridge are shown by open symbols. (c) Fitted allele frequency clines for different loci in 1987 (solid lines) and 2005 (broken lines). Distance across *X*-axis is in kilometers, zero point is at Öresund Bridge. Separate plots for individual loci are in the ESM, Fig. 1

Samples and characters

We collected samples of *Mytilus* from ten shallow-water sites (< 3 m depth, usually about 0.5 m) from the Öresund area, repeatedly in 1987 and in 2005. Additional data on two localities far from the hybrid zone were included to represent pure parental populations, i.e. Hjerting, North Sea and the Gulf of Gdansk, inner Baltic; the temporal samples from the Gulf of Gdansk were collected from two sites 20 km apart (Table 1, Fig. 1a). Material was genotyped at the six polymorphic allozyme loci *Lap* (coding for leucine aminopeptidase), *Idh-1* (isocitrate dehydrogenase), *Odh* (octopine dehydrogenase), *Pgm* (phosphoglucomutase), *Gpi* (glucose-6-phosphate isomerase)

and *Est-D* (carboxylesterase), using starch gel electrophoresis following the methodology in Väinölä and Hvilsom (1991) and Murphy et al. (1996). The loci are generally multiallelic and are strongly differentiated between the two species, while not completely diagnostic. Alleles at each locus were binned into *M. edulis* and *M. trossulus* specific compound alleles as in Väinölä and Strelkov (2011); these are called the *E* and *T* alleles, respectively.

Table 1 Sample information

Sampling site (sample code)		Coordinates	Distance	Sample size 198
1.	Hjerting, Denmark (Hjer)	55°28′07″N 08°22′30″E	-700	129 (1987 only)
2.	Halmstad, Sweden (Halm)	56°38′01″N 12°47′44″E	- 133.8	120/85
3.	Hittarp, Sweden (Hitt)	56°05′29″N 12°38′46″E	- 56.5	108/79
4.	Landskrona, Sweden (Lkr)	55°52′16″N 12°49′04″E	- 31.2	178/75
5.	Vikhög, Sweden (Vhg)	55°43′42″N 12°55′33″E	-16	110/52
6.	Kastrup, Denmark (Kast)	55°38′23″N 12°39′18″E	- 7.7	214/96
7.	Limhamn, Sweden (Limh)	55°35′24″N 12°55′17″E	- 0.8	120/123
8.	Brøndby, Denmark (Bro)	55°36′48″N 12°25′07″E	13.heinä	60/129
9.	Skanör, Sweden (Ska)	55°25′12″N 12°49′58″E	16.huhti	109/116
10.	Kämpinge, Sweden (Kamp)	55°23′50″N 12°58′58″E	51.6	107/90
11.	Ystad, Sweden (Yst)	55°25′46″N 13°49′22″E	104.6	206/78
12.	Gulf of Gdansk, Poland (Gda)	54°26′53″N 18°34′34″E	700	140 (1987)
		54°35′14″N 18°44′17″E	700	100 (2005)

Distance along the transect is measured in kilometers from the Öresund Bridge; negative values are on the Kattegat side, positive values on the Baltic side. Sample size is the average number of animals studied across loci

Cline position and shape

The location and shape of the clines, and the degree and dynamics of hybridization were assessed in a framework of theoretical models of hybrid zone structure. Distances between localities were measured in kilometers along a transect line from Kattegat to the Baltic through the Öresund. The zero reference point was placed at the Öresund Bridge. Positions of the remote Hjerting and Gdansk references were both arbitrarily set at 700 km from the Bridge. The transition of allele frequencies across distance was modeled by symmetrical sigmoid clines using the program NLREG 6.5 (Sherrod 2008). The sigmoid cline $X = 1/(1 + \exp[-4(d - c)/w])$ is characterized by two parameters: the width of the cline (w; the inverse of the maximum slope of the cline) and the midpoint or center (c) of the cline (e.g., Szymura and Barton 1986). Among various models of genetic clines, the sigmoid is the simplest one, with the minimum number of parameters, and was therefore considered appropriate to deal with the current data set of a small number of samples. The clines were fitted to the *T* frequencies that had been scaled to the interval [0, 1] between the Hjerting and Gulf of Gdansk references, considered as "pure" *M. edulis* and Baltic *M. trossulus*, respectively. In addition to these estimates of the position and shape of the clines of individual loci at each two points of time, we similarly characterized the overall multilocus clines by fitting to the average scaled frequencies of all loci.

Cline coincidence (centers at the same position) and concordance (equal cline widths) among loci in each temporal survey were explored by the polynomial fitting method of Szymura and Barton (1986). Scaled *T* frequencies at each of the six markers were plotted against the average frequency across loci (X). If the clines coincided, all the points would lie on the diagonal, whereas if not, the points would be scattered around the diagonal. The patterns in such scatter were evaluated by fitting a polynomial $p_T = X + 2\alpha X(1 - X) + 2\beta X(3X - 2X^2 - 1)$ where a positive α indicates a

deviation towards *T* alleles (i.e., cline deviates from others towards the Kattegat) and positive β indicates a cline narrower than the average. NLREG 6.5 was used for this analysis.

The significance of temporal changes in cline position and shape over two decades was tested by comparing the locus-wise point estimates of *c* and *w* from 1987 to 2005 using paired Student's *t* test, after verifying the assumptions of the test, using PAST (Hammer et al. 2001). Also, temporal allele frequency differences at each locality and locus were separately assessed by exact tests of genic differentiation (Genepop v. 3.4, Raymond and Rousset 1995) applying a Bonferroni multiple test correction (Rice 1989).

Hybridization and dispersal

Tests for single-locus genotypic equilibria were performed using Genepop (see ESM Table S1 for details). The extent of hybridization in populations was assessed from two-locus genotypic structure by comparing the observed genotypic distributions in the samples to two extreme reference situations, i.e. the distributions expected in panmictic local populations on the one hand, and in non-hybridizing mixtures of the two parental taxa on the other. Two parallel approaches were used. First, the non-random association of genotypes was assessed in terms of the inter-locus genotypic correlation coefficient R' (a standardized composite digenic association measure, analogous to pairwise linkage disequilibrium). R' values were calculated as weighted averages over pairs of the four most strongly differentiated loci *Est-D, Gpi, Odh* and *Pgm*, and the corresponding maximum values R_{max} (as expected in hypothetical mixtures of non-interbreeding "pure" taxa) were obtained using the reference population frequencies and the average scaled *T* allele frequencies (= *X)* as an estimate of the population mixing proportion (see Nikula et al. 2008). In a panmictic local equilibrium, population R' is expected to be zero. This approach was used for Öresund *Mytilus* data also in Väinölä and Hvilsom (1991) and Väinölä and Strelkov (2011). Second, as an alternative method we used the newly proposed "degree of genetic mixing" parameter m_d = $\mathrm{Var}(P_i)/(P(1-P))$, where *P* represents the overall proportion of *M. trossulus* genes in a population and P_i represent the proportion of *M. trossulus* genes in the genome of the *i*th individual (Kalinowski and Powell 2015). The P_i's and *P* were obtained using the Bayesian genotypic assignment method implemented in the program STRUCTURE 2.3.4. (Pritchard et al. 2000; program settings: two-population admixture model with default parameters, correlated allele frequencies, 100,000 iterations after 10,000 burn-ins). Theoretically m_d will vary from zero (no hybrids) to one (all individuals in the population have the same amount of *M. trossulus* ancestry). The two methods are largely analogous, but for the estimation of m_d the relative population contributions *P* were obtained implicitly from the Bayesian analysis (all individuals of the dataset were input in the analysis with no prior information on their population identity), whereas the expectations for R' are based on explicitly chosen parental population frequencies. The m_d method was applied to the dataset of all six loci analyzed. To present the results in a similar manner with that of R', we used $1 - m_d$ for the graphical plot.

Under the conventional model of a stable hybrid zone, the width of a sigmoid cline *w* is determined by a balance between the opposing effects of selection (*s*) and dispersal (σ), which would tend to diversify and homogenize geographical populations along the transect, respectively (Barton and Hewitt 1985). The deficit of hybrid gametic haplotypes versus random expectation (measured here as $R^* = R'/R_{max}$) is a crude estimate of selection against hybrids along the cline (i.e., the extent to which hybrids are less readily generated or maintained) (Szymura and Barton 1986; Sotka and Palumbi 2006). According to Szymura and Barton (1986), in the center of a cline $\sigma \sim \frac{1}{2}w(rR^*)^{0.5}$, where σ is the dispersal distance (standard deviation of parent–offspring distance along the transect) and *r* is the recombination rate among loci ($r = 0.5$ for unlinked loci, as in our case). To assess the dispersal distance in mussels, we substituted estimates of R^* and *w* from the overall clines in this formula.

Comparison of age cohorts

The sampled populations represent mixtures of multiple annual cohorts, derived from spat settled from larvae over several years; this constitutes a source of noise and potential bias when assessing temporal population differences, when both the sources of recruiting stocks (origin of larvae along the steep cline) and the relative representation of the cohorts may vary. To control for the effect of settling cohort variation, a subset of samples was analyzed in more detail, divided into age classes. This subset involved the 1987 and 2005 samples of the four intra-zone sites Limhamn, Skanör, Vikhög and Kastrup. An operational age of mussels was determined by counting the "growth rings" (rings of winter growth delay) on the outer shell surface, as in Sukhotin et al. (2007). Individual genotypes were characterized by the number of *T* alleles at the four loci *Pgm, Gpi, Odh* and *Est* (varying in the interval 0…8). A set of two-factor ANOVAs was performed to assess the influence of age and sampling period on the number of an individual's *T* alleles, at each locality separately, using PAST. As the age class distributions in 1987 and 2005 were not the same, marginal age classes were sometimes pooled to adjacent ones to create comparable data matrices. The square root transformation of the data $(N_T + 0.5)^{0.5}$ was applied to meet the assumptions of ANOVA.

Results

All loci showed strong allele frequency differences between the reference *M. trossulus* population of Gdansk from the inner Baltic and *M. edulis* of Hjerting from the North Sea: the absolute frequency differences were 43% at *Idh*, 52% at *Lap*, 62% at *Odh*, 86% at *Pgm*, 95% at *Gpi* and 97% at *Est*. The genetic constitution of Ystad samples, 100 km into the Baltic from the Öresund, was very similar to that of the Gulf of Gdansk while that from Halmstad, 100 km into the Kattegat, still had ca. 20% average *M. trossulus* contribution. Other samples had intermediate allele frequencies (Table 2). Overall, the allele frequencies changed gradually along the transect through the strait, with the most drastic changes occurring in the central and southern (i.e., the Baltic side) parts of it. The monotony of the change was, however, disturbed by the Vikhög–Limhamn samples, where the trend was locally reversed (Fig. 1a, see ESM for graphical plots of the locus-wise allele frequencies along the transect).

Table 2 Frequencies of composite *T* alleles and estimates of inter-locus disequilibrium *R′* (averaged over pairs of loci) in samples from different decades, and of parameters of sigmoid clines fitted to individual loci (*c* cline midpoint, *w* cline width)

	Pgm		***Odh***		***Gpi***		***Est***		***Idh***		***Lap***		***R′***	***R′***
	2005	**1987**	**2005**	**1987**	**2005**	**1987**	**2005**	**1987**	**2005**	**1987**	**2005**	**1987**	**2005**	**1987**
Hjer	0.00	–	0.01	–	0.05	–	0.02	–	0.40	–	0.00	–	–	0.00
Halm	0.15	0.14	0.06	0.09	0.20	0.30	0.14	0.21	0.45	0.41	0.06	0.08	0.02	0.02
Hitt	0.16	0.20	0.11	0.13	0.25	0.36	0.22	0.37	0.38	0.54	0.11	0.18	0.04	0.04
Lkr	0.20	0.31	0.09	0.25	0.28	0.37	0.31	0.40	0.41	0.51	0.10	0.18	0.05	0.12
Vhg	0.50	0.73	0.33	0.43	0.62	0.81	0.52	0.77	0.49	0.72	0.28	0.46	0.39	0.20
Kast	0.19	0.45	0.13	0.30	0.29	0.57	0.25	0.50	0.59	0.57	0.16	0.25	0.05	0.21
Limh	0.45	0.65	0.25	0.42	0.52	0.75	0.40	0.74	0.56	0.63	0.23	0.44	0.22	0.14
Bro	0.62	0.70	0.50	0.54	0.79	0.72	0.71	0.71	0.65	0.69	0.36	0.33	0.28	0.10
Ska	0.73	0.76	0.57	0.51	0.84	0.81	0.78	0.85	0.69	0.76	0.44	0.43	0.18	0.1
Kamp	0.75	0.89	0.50	0.55	0.84	0.94	0.80	0.94	0.76	0.79	0.60	0.54	0.14	0.02

Yst	0.89	0.89	0.73	0.60	0.95	0.97	0.96	0.96	0.76	0.81	0.49	0.48	0.10	0.10
Gda	0.91	0.87	0.61	0.63	0.95	0.99	0.95	0.99	0.75	0.83	0.54	0.52	0.06	0.00
c (km)	- 5.7	- 26.8	- 2.4	- 21.9	- 8.7	- 35.2	- 2.5	- 34.2	##### #	- 16.7	- 4.9	- 31.8		
w (km)	99.4	97.7	70	116.6	113.9	175.7	129	163	74.6	141.5	80	139.1		

See Table 1 for average sample sizes

Plotting (scaled) individual marker frequencies against the average frequency of *T* alleles and fitting a polynomial relationship demonstrated good coincidence and concordance among most clines, but *Idh* made a possible exception (Fig. 2). At both time points, the *Idh* clines appeared to be located more towards the Baltic than the others, with an offset of about 10–15% of the overall cline width.

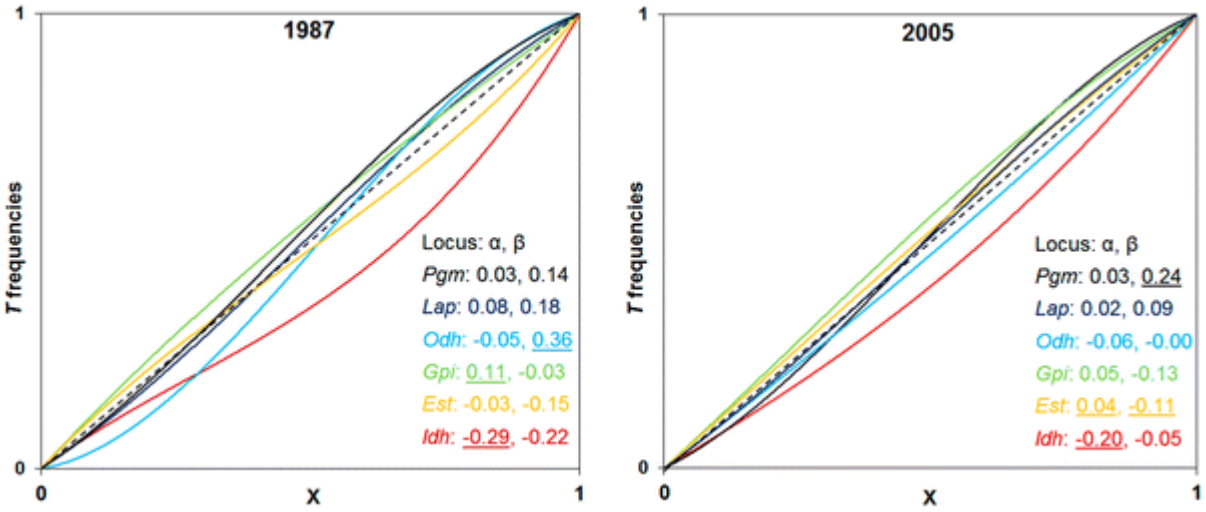

Fig. 2 Comparison of cline concordance at different loci in two temporal surveys. The *T* allele frequency at each locus was scaled to (0, 1) between Hjerting and Gdansk reference samples and plotted against the scaled average frequency *X*. Cubic polynomials forced to pass the (0, 0) and (1, 1) endpoints were fitted to the data. For each time point, estimates of the deviation of cline midpoint (α) and relative cline width (β) in relation to the mean cline at that time are presented. Values significantly different from zero at $p = 0.05$ [two-sided *t* test implemented in NLREG, Sherrod (2008)] are underlined. The dashed diagonal represents the overall cline. Individual curves and parameter values are indicated

Temporal changes in cline position and shape

In temporal comparisons of clines between the 1980s and 2000s, the frequencies of *T* alleles at most loci had dropped down in all populations north of the zero reference point at the Öresund Bridge (i.e., on the Kattegat side; Fig. 1a, b and Fig. 1 of ESM). For the Limhamn, Kastrup and Vikhög populations in the central part of the clines, the magnitude of change was 30–50% in terms of scaled frequencies (Table 1, Fig. 1 of ESM). Temporal changes in allele frequencies were significant ($p < 0.01$) for all Öresund localities north from the Bridge (Hittarp, Landskrona, Vikhög, Kastrup, Limhamn) as well as for Kämpinge on the Baltic side, and insignificant for all other localities ($p > 0.1$; exact tests over all loci).

The temporal genetic changes can better be illustrated in terms of the parameters of the fitted sigmoid clines. In 1987, the estimated midpoints of individual clines were located within a distance of - 5.2 km (*Gpi*) to -16.7 km (*Idh*) from the Bridge (mean ± SE = -27.8 ± 7.4 km). In 2005, the midpoint estimates ranged from -8.7 km (*Gpi*) to 5.7 km (*Idh*) (mean ± SE = 3.1 ± 4.9 km; individual cline parameters in Table 2). The overall change is significant at $p = 0.0001$

(paired *t* test). The estimates of individual cline width in 1987 were from 98 km (*Pgm*) to 176 km (*Gpi*), mean ± SE = 138.9 ± 12.9 km, and in 2005 from 70 (*Odh)* to 114 km (*Gpi*), mean ± SE = 94.5 ± 10.6 in 2005; the change is significant at $p = 0.01$, paired *t* test. Thus, the midpoint of the multilocus cline had shifted by about 25 km from the central part of the Öresund transect to the close proximity of the Bridge in the southern end of the strait, and the average cline width had narrowed by about 30% (Fig. 1c).

Genotypic structure, hybridization and dispersal

The estimates of the average inter-locus disequilibrium values R' in individual samples at the two time points are presented in Fig. 3a, along with the curve of the expected maximum value in the corresponding non-interbreeding mixture of parental populations. The average of the R' estimates across the nine populations with mixed ancestry (excluding Ystad, Gdansk and Hjerting) was 0.11 in 1987 and 0.15 in 2005. In populations closest to the estimated cline center (i.e., with a scaled *T* frequency close to 0.5), the R' values were up to 30–50% of the maximum (Kastrup, 1987 $R' = 0.21$, or 39% of the maximum; Limhamn 2005 $R' = 0.22$, $R^* = 30\%$; Vikhög 2005 $R' = 0.39$, $R^* = 52\%$). The overall rise of disequilibria, or the drop in the extent of hybridism, is similarly illustrated in terms of the degree of genetic mixing parameter m_d (Fig. 3b): in the corresponding samples, average values of $1 - m_d$ over the nine samples were 0.22 in 1987 vs. 0.27 in 2005, while the maximum values were 0.28 (1987) and 0.37−0.50 (2005). Substitution of R^* values as an estimate of selection and the estimated width w of the overall clines (139 km in 1987 and 94 km in 2005) in the equation that relates selection to hybrid zone width would give estimates of dispersion distances σ of 31 km (1987), and 18−24 km (2005), respectively.

Single-locus heterozygote deficiencies were also generally observed. Statistically significant deviations were mostly found in the 2005 samples from the vicinity of the Bridge (Vikhög–Brøndby area) (ESM Table S1).

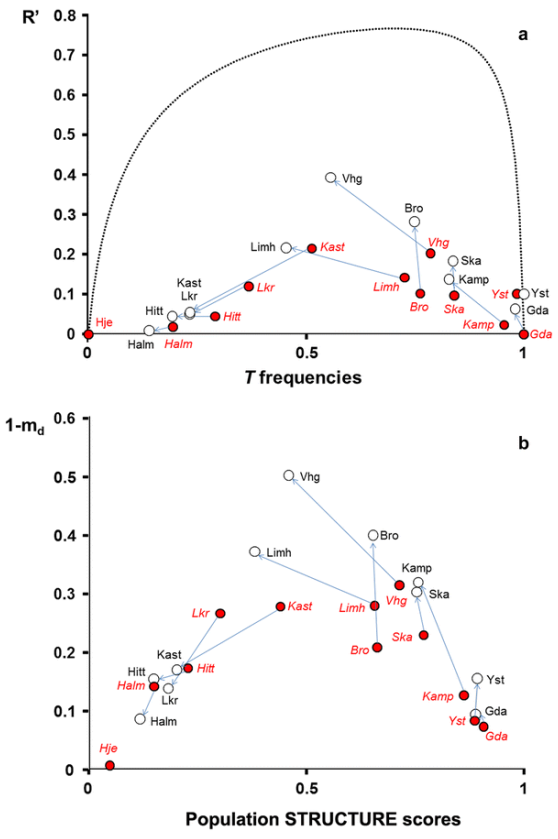

Fig. 3 Estimates of the extent of hybridization/mixing in temporal samples from 1987 to 2005. (a) Estimates of the standardized average pairwise inter-locus disequilibrium *R′*. *X*-axis—scaled average of the *T* allele frequency for the four loci *Est*, *Gpi*, *Odh* and *Pgm*. The curve shows the maximum possible disequilibrium, expected in hypothetical non-interbreeding mixtures of the putative parental populations (see Nikula et al. 2008). **(b)** Estimates of "the degree of genetic mixing parameter" m_d of Kalinowski and Powell (2015). *X*-axis—proportion of *M. trossulus* genes over six loci in a sample estimated by STRUCTURE (population STRUCTURE score). *Y*-axis − 1− m_d. Data from 1987 are shown by filled circles, from 2005 by open ones. Temporal samples from the same locality are connected by arrows

Genetic differences between age cohorts

The number of growth rings in mussels in our collections from Limhamn, Skanör, Vikhög and Kastrup varied between 2 and 11, and the different samples differed in respect to the age class composition (ESM Fig. S2). In the ANOVA analyses performed at each site separately (ESM Table S2), temporal differences between decades in the genetic constitution (i.e., in average individual *T* allele frequency across loci), independent of age, were highly significant at the two sites Kastrup and Vikhög, but not at the two others. Effects of age on the genetic constitution were significant at one of the three sites, Limhamn ($p = 0.002$), where in turn no long-term temporal difference was detected. At this site only, a general increase of *T* frequencies with age was seen in both temporal samples (Pearson's correlation between the number of rings and mean number of *T* alleles in genotypes of an age class $r = 0.96$, $df = 4$, $p = 0.01$ for 1987, $r = 0.90$, $df = 4$, $p = 0.01$ for sample from 2005). No significant interaction effect between age and collection time was found at any of the sites, indicating that genetic differences between age cohorts within samples hardly could influence the temporal comparisons.

Discussion

Our genetic data disclose a typical hybrid zone in the Öresund mussels—a sharp and relatively narrow multilocus cline with shortage of intermediate genotypes in the center—in line with the previous, less extensive allozyme studies from the same strait (Väinölä and Hvilsom 1991; Väinölä and Strelkov 2011). The approximate width of the Öresund transition is 100 km, the relative inter-locus disequilibrium estimates $R^* = R'/R_{max}$ are 0.3–0.5 in the center of the cline (a crude estimate of the strength of selection against hybrids in the framework of the tension hybrid zone model, see below), and the genetically estimated dispersal distance is about 25 km. Although the dispersion estimate may appear quite small for blue mussels whose larvae spend several weeks in the plankton before settlement (Bayne 1976), it is well in line with other, independent genetic and non-genetic estimates of dispersion in *Mytilus* (30–50 km, Gilg and Hilbish 2003, 20–30 km, Becker et al. 2007), in particular with those obtained by modeling larval drift in the Kattegat and Öresund regions (10–30 km, Stuckas et al. 2017). However, as our samples represent only shallow-water populations, the potential vertical dimension of the hybrid zone organization remains unknown. It should also be pointed out that the Öresund hybrid zone represents only a segment of the complex connection between the Kattegat and the Baltic, which encompasses also a parallel transition zone across the other Danish Straits. Data from that zone and the southwestern Baltic coast (using other sets of marker loci) show a more gradual genetic transition, nearly twice broader than in the Öresund and with weaker intra-cline disequilibria (Zbawicka et al. 2014; Stuckas et al. 2017), in line with the less steep environmental transition. Indicatively, even our samples from the Gulf of Gdansk were not identified as truly equilibrium and "pure" representatives of the Baltic *M. trossulus* in STRUCTURE and m_d analyses but had 9–11% contribution of alien genes and non-zero m_d values (Fig. 3b).

Interpretation of the hybrid zone

Since the very discovery of the genetic distinction of Baltic vs. North Sea *Mytilus* (Theisen 1978; Bulnheim and Gosling 1988; Varvio et al. 1988), the nature of the differences and of the transition zone has been a matter of some controversy (see, e.g., Riginos and Cunningham 2005; Väinölä and Strelkov 2011). One question is about the geographical extent of the Baltic hybrid zone: some authors (e.g., Riginos and Cunningham 2005) understand that it would encompass the entire Baltic Sea population because the Baltic *M. trossulus* bears the mitochondrial DNA from *M. edulis* (Wenne and Skibinski 1995; Rawson and Hilbish 1998) and high frequencies of *M. edulis* specific alleles also at a number of otherwise diagnostic nuclear DNA markers (Borsa et al. 1999; Riginos et al. 2002). This interpretation follows a fashion of applying the term "unimodal hybrid zone" to populations of mixed taxonomic ancestry that are in genetic equilibrium (probably traceable to Jiggins and Mallet 2000). In the alternative concept that we follow, the Baltic *Mytilus* hybrid zone refers only to the transition zone between *M. edulis* of the Kattegat and the homogenous populations of 'the Baltic mussel' *M. trossulus* within the inner Baltic, each inferred to represent an effective equilibrium state despite being introgressed (see Väinölä and Strelkov 2011 for further discussion).

Another topic of contention has been whether allozyme data are representative of the genomic composition (systematic background) of the *Mytilus* populations in the region and illustrative of the process of genomic mixing. Early results suggested that the level of differentiation in allozymes is higher than in the initial few nuclear DNA markers studied, and prompted a suggestion that allozymes could be directly affected by salinity-related selection (Riginos et al. 2002; see also Johannesson et al. 1990). If so, the position and shape of the allozyme clines could differ from the overall genomic cline caused by reproductive barriers between the hybridizing species. The set of loci compared by that time was, however, inconclusive (Bierne et al. 2003), and newer studies involving far greater numbers of nuclear DNA markers, mostly SNPs, suggest that on average they are no less differentiated than the allozyme set (Bierne et al. 2003; Stuckas et al. 2009; Zbawicka et al. 2012, 2014; Fraïsse et al. 2016). While so far there have been no proper studies on the concordance between allozyme and non-allozyme clines within the Öresund or the other transition areas, notable discrepancies are found among the individual nDNA markers, suggesting outliers rather within that set of characters (Stuckas et al. 2009, 2017). To note, in our data set the geographical pattern at the putatively salinity selected locus *Lap* (Hilbish and Koehn 1985; Väinölä and Hvilsom 1991) did not differ from that at the other loci (save *Idh*). By this time we see no evidence to suggest that the multilocus allozyme cline would not be representative of the overall genomic structure of the Öresund hybrid zone.

Temporal dynamics of the zone

Mechanisms that theoretically can account both for a long-term stability and for a spatial dynamics (movement) of hybrid zones have been outlined by Barton and Hewitt (1985) and Barton and Gale (1993). Zones could be maintained by a balance between selection against hybrids (a tension zone) or against parents in their non-native environments (an ecotonal zone), and dispersal of parental genotypes into the zone. Controlled by these forces, tension zones will become spatially associated with areas of lower population density ("density troughs") or with other barriers to dispersal. Ecotonal zones are associated with ecological gradients where the relative fitnesses of the hybridizing species change. Changes in population density and position of physical dispersal barriers, and changes in ecological gradients can cause changes in the position of hybrid zones. In nature, both endogenous selection against hybrids and exogenous selection against parents could potentially act in the same hybrid zones (Barton and Gale 1993), and this has also been suggested for the blue mussel zone in the Öresund (Väinölä and Hvilsom 1991): the zone would likely be affected both by selection against hybrids and by selection against parents, first of all by impeding

dispersal of larvae entering a salinity different from their native habitat. *M. edulis* of the Kattegat and *M. trossulus* of the inner Baltic are adapted to very different salinity regimes and even adults hardly survive reciprocal translocations (Theisen 1978; Johannesson et al. 1990) while larvae are generally thought to be even less tolerant than adults (Bayne et al. 1976).

The main result of our study is that between the mid-1980s and mid-2000s the midpoint of the hybrid zone in the Öresund shifted from the center of the transect along the strait about 25 km, south towards its Baltic side, to the Drogden Sill area, where the Öresund fixed link was built in 1995–1999. Correspondingly, *M. edulis* expanded within the Öresund while the distribution of *M. trossulus* retreated towards the Baltic. In the theoretical framework, such a shift could have either been invoked by a shift in the environmental gradient, such as a salinity rise in the area, or by changes in mussel density and positions of physical barriers to mussel dispersion in the Öresund.

Hybrid zone movement due to environmental change

Environmental monitoring data (annual summer bottom salinity anomalies, Lindegren et al. 2010) do not show any general increase in salinity in the Öresund from the 1980s to 2000s. A few strong inflow events that brought saline water into the Baltic, sporadic by their nature, occurred through the period, the two major ones in 1993 and 2003, both in the winter time. Yet the frequency of such events through 1986–2008 was lower than through the preceding decades (Mohrholz et al. 2015). Thus, we cannot readily relate changes in mussel populations to changes in salinity. Theoretically, the long-term changes of another key physical parameter, temperature could affect mussels. Apart from the low salinity, the inner Baltic Sea is characterized by relatively low water temperatures (HELCOM 1996), and this could be a factor affecting mutual distributions of mussel species in the Danish Straits. As everywhere in the North Atlantic, climate was steadily warming both in the Southern Baltic and in Kattegat through the 1980s–2000s (annual mean sea surface temperatures raised 0.6 °C on average, MacKenzie and Schiedek 2007; Lima and Wethey 2012). The temperature rise in the Danish Straits was accompanied with decreasing nutrient inputs, which had a negative synergetic effect on the biomass of phytoplankton (food for mussels) that decreased twofold (Henriksen 2009). Although both *M. edulis* and *M. trossulus* in the wider framework are boreal–arctic species, physiological experiments on North American mussels have suggested that *M. trossulus* is indeed more cold-adapted than *M. edulis* (Hayhurst and Rawson 2009; Rayssac et al. 2010; Fly and Hilbish 2013). Along the Northwest Atlantic coast the southern limit of *M. trossulus* is in the eastern Gulf of Maine, and it is completely substituted by *M. edulis* to the south of that (Hayhurst and Rawson 2009). Sea surface temperatures in this area are similar to those in the Kattegat: the long-term annual mean temperatures in Boothbay Harbor, Eastern Maine and in Skagen, Northern Kattegat are 8.5 and 8.9 °C correspondingly, while the long term from January to March means are about 2.5 °C at both points (data from Drinkwater and Petrie 2011; MacKenzie and Schiedek 2007). Geographical shifts in position of other hybrid zones between *Mytilus* species have also been reported and attributed to climate warming (Hilbish et al. 2010, 2012). Elevated winter temperatures have a negative effect on the physiological performance of both *M. edulis* and Baltic *M. trossulus* (Honkoop and Beukema 1997; Waldeck and Larsson 2013). Our hypothesis is that elevated water temperatures in a situation of limited food resources through 1980–2000 would have more severely affected the cold-adapted *M. trossulus* than *M. edulis* in Kattegat and the Öresund, giving and advantage to *M. edulis* to expand into the Öresund, whereby the distribution of *M. trossulus* retreated deeper into the cool Baltic Sea.

Hybrid zone movement due to changes in dispersal, under the Bridge

The Drogden Sill where the midpoint of the hybrid zone was located in 2005 is the area with the highest mussel densities in the Öresund. In the early 1990s, an enormous mussel stock covering an area of 128 km^2 with average biomass 7.1 kg/m^2 was estimated to be present. These mussels would

have been able to consume nearly all phytoplankton from the water passing the area (Haamer and Rodhe 2000). Therefore, in respect to benthic mussel abundance, the Drogden Sill area is not a density trough that could attract a (tension) hybrid zone but rather the worst place for a predicted location of the clines, from the adult life stage point of view. From the larval perspective the situation is, however, different. The Sill is the region of the steepest salinity changes in the Öresund, and considered as the true oceanographical boundary of the Baltic Sea (Fischer and Matthäus 1996). It has been hypothesized that most of the invertebrate larvae imported from Kattegat and the Öresund into the sill area die from the osmotic stress (Thorson 1946; Nordenberg 1980). Further the mussel bed itself could restrict the movement of mussel larvae through the sill area because filtering adult mussels also consume and destroy conspecific larvae (Davenport et al. 2000). In such a context, the Drogden Sill could be seen as a barrier to mussel dispersion, and therefore a place for a center of a hybrid zone. Nevertheless, we are not aware of long-term demographic changes that could have invoked the shift of the hybrid zone from central stretch of the strait transect into the sill area.

At the same time, the potential influence of the changes caused by the new Öresund fixed link should be considered. The construction of the link may have had both negative and positive effects on benthic mussels: some previously inhabited substrate was irreversibly destroyed while new artificial substrate for mussel fouling, e.g., pillars of the bridge, appeared (Gray 2006). While it has been declared that the link did not change the water flow through the Strait (Gray 2006), we suspect that minor changes in the hydrodynamics and (or) in the benthic habitat caused by the construction have further hampered the larval dispersion through the sill area and (or) reduced the reproductive output of the local mussel stock. These changes would have made the Drogden Sill a stronger barrier to dispersion, capable of "attracting" and trapping a hybrid zone from its previous position further north in the strait.

Changes in zone structure

Concomitant with the change in the zone position, the curve fitting approach suggested that the cline widths also decreased (about 30%), whereas the relative inter-locus disequilibria R' that describe the local genotypic structure seem to have got stronger. Using other terms, the latter change can be viewed as a decrease of "hybridizm" or of interspecific genomic mixing in the zone populations (Fig. 3b). In the basic hybrid zone equation, the three parameters w, R^* (representing selection) and σ (dispersal) are intertwined; a decrease of cline width would either result from a decrease in dispersal or from stronger selection against hybrids (reflected in higher R' or lower m_d estimates). In Öresund, an effective reduction of dispersal could indeed have resulted from the same (hypothetical) changes in hydrography that caused the movement of the zone to the sill position and strengthened the local physical dispersal barrier, trapping the zone in new location. [Such change of a cline width in fact is expected for a tension zone that has recently moved; indeed the "tension hybrid zone" model was named due to the expected tendency of such a zone to move so as to minimize its length (Barton and Hewitt 1985)]. Such a change can also be viewed as an increase of the average (exogenous) selection against the parental genotypes entering the zone area.

An increase of R' and reduction of m_d as a result of a change in the basic dynamics of hybridization would appear inherently unexpected in the Öresund zone. In principle, hybrid zones themselves are expected to evolve, e.g., through reinforcement towards stronger reproductive isolation if selection is strong enough, or conversely towards erosion of reproductive barriers. Nevertheless, under the concept of a long-term post-glacial parapatry of *M. edulis* and *M. trossulus* in the Baltic Sea (Väinölä and Hvilsom 1991; Väinölä and Strelkov 2011), we have assumed that this hybrid zone existed for millennia and is a stable formation, unlikely to change substantially through a two-decade interval. At all events, the observed changes (narrowing clines, increased disequilibria) appear consistent with a reinforcement hypothesis. A suggested mechanism to strengthen

interspecies reproductive barriers in hybrid zones on a short time frame is through establishment of associations between barrier loci that influence reproductive isolation and loci subject to exogenous selection, when previously uncoupled clines come together (Barton and De Cara 2009; Bierne et al. 2011; Abbott et al. 2013). Clines at (previously) independent endogenous barrier loci are themselves expected to become coupled with each other and trapped in areas of lower population density. Cline positions may move for various reasons (cf. above) and while independently governed clines (e.g., exogenous and endogenous) meet they will be coupled and likely become geographically stabilized by the exogenous factors (environmental gradients or other dispersal barriers). This effectively increases the number of loci contributing to reproductive isolation at the new joint position, and increases the disequilibria also among the previously associated loci (Barton and De Cara 2009; Bierne et al. 2011; Abbott et al. 2013). We do not have any evidence that the (few) studied loci would themselves be involved in selection. Meanwhile, through their systematic background most of them are likely to be associated with the genome component subject to endogenous isolation. It could be speculated that the spatial perturbation of the hybrid zone and move to a reorganized oceanographic break at the Drogden Sill was also associated with establishment of new associations, e.g., between endogenous barrier loci and unobserved clines (loci) under exogenous selection resting in the Sill area.

Comparative aspects of Baltic–North Sea transitions

The Öresund and the Danish Straits as a whole make a transition zone, an area of drastic changes in hydrography and in the structure of marine biodiversity (Bonsdorff 2006). It is also an area of genetic changes in populations for taxa whose distributions span over the environmental gradient (Johannesson and André 2006). While we recorded a recent shift in the position of the *Mytilus* genetic clines towards the Baltic direction, the question arises whether similar shifts have occurred in the genetic clines of other taxa as well. Here it should be noted that the documented changes fall in various categories, with regard to the age of the differences and to the forces that are thought to maintain them (e.g., exogenous vs. endogenous selection, historical contingency). In an earlier review of genogeographic marker data of the Baltic Sea macro-organisms, genetic breaks in the vicinity of the Danish Straits were found in at least 14 of the 24 species assessed (Johannesson and André 2006; Johannesson et al. 2011). In most cases such genetic differences across the zone are intra-specific and have been established post-glacially, since the colonization of the Baltic. They have been attributed to severe historical bottlenecks of the Baltic Sea populations or to a specific selection regime in the cool and brackish Baltic Sea (Johannesson and André 2006). The genetic clines that link the differentiated populations of various taxa tend to coincide in the same areas; these have been demonstrated both in the Belt Sea (e.g., Atlantic cod *Gadus morhua* L., turbot *Scophthalmus maximus* L., Nielsen et al. 2003, 2004) and in the Öresund (e.g., diatom *Skeletonema marinoi*, Sjöqvist et al. 2015). Second, more recent genome scale surveys in fishes have revealed genomic regions with increased level of differentiation between Kattegat and inner Baltic on a background of general homogeneity. They are interpreted as directionally selected regions in which variability is governed by salinity and (or) temperature (e.g., Guo et al. 2015; Berg et al. 2015; Barrio et al. 2016). In contrast to these cases of putatively primary, post-glacial differentiation, the clines in *Mytilus* marker loci do not represent differences that arose in situ, but in the first place represent secondary intergradation of old lineages.

Apart from *Mytilus*, the best characterized genetic transition in the Öresund and the closest point of comparison is in the Baltic clam *Macoma balthica*, which likewise represents a secondary contact of two anciently diverged lineages (Nikula et al. 2008; Luttikhuizen et al. 2012), and also features a similar life history. However, the zone profiles do not exactly coincide. In data from 2005, the midpoint of the notably wide (657 km) multilocus allozyme cline in shallow-water *Macoma* populations was estimated to have been 20 km further south from the midpoint of the *Mytilus* hybrid zone at the same time (Nikula et al. 2008). Similarly, the main transition in the

(post-glacial) genetic structure of cod occurs within the Baltic basin, displaced from the oceanographic break (Nielsen et al. 2003).

In all, we recorded a recent shift in the position of the blue mussel hybrid zone towards the Baltic direction, and a change in the cline shape. The primary cause of the change was not unambiguously resolved, but two hypotheses were posed: (1) the Baltic *M. trossulus*, as the more stenothermal of the taxa was pushed out from the Öresund by elevated water temperatures; (2) The construction of the Öresund fixed link hampered the gene flow from *M. trossulus* of the inner Baltic towards the Öresund which permitted *M. edulis* (and its genes) to expand. If it is primarily about temperature, the warming climate could affect other Baltic Sea residents in a similar manner since they are historically adapted to low temperatures. If it is about physical effects of the Öresund fixed link, effect would primarily be expected for species with *Mytilus*-like life histories, i.e., benthic invertebrates with larval dispersal.

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Supplementary material 1

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